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Seroepidemiology of *Coxiella Burnetii* in commercial dairy herds in northeast of Iran

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Abstract

Q fever is an important zoonotic disease caused by infection with Coxiella burnetii. Limited information is available concerning theseroprevalence of Coxiella burnetii in Iran.A serological survey was conducted to describe the seroepidemiology of Coxiellaburnetii infection in dairy cattle in Khorasan Razavi province located in northeast of Iran. 246 dairy cattle from 19 commercial dairy herds that were distributed in 9 counties were selected. Blood samples were assayed for antibody to Coxiella burnetii using CHEKIT Q fever ELISA kit. Seroprevalence of Coxiella burnetii at animal and herd level was 22.3 (95% CI: 17.1-27.6) and 78.9 (95% CI: 60-97) percent, respectively. Coxiella burnetii was distributed all over the province. The proportion of seropositive animals ranged from 0 - 62.5%in the studied herds and it was different significantly in various regions (P=0.001).Parity and herd size were not associated with seroprevalence. High prevalence of antibody against Coxiella burnetii in the cattle population of the study area implies zoonotic and economic importance. More investigations on the other reservoirs and human (especially at risk population) are necessary to make epidemiologic feature of Coxiellosis clear.

Keywords: seroepidemiology, *Coxiella burnetii*, dairy cattle, Iran

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Introduction

Q fever is a zoonoses caused by Coxiella burnetii, a small obligatory intracellular gram-negative bacterium that is a very environmentally stable agent (Romich, 2008). The bacterium is distributed globally except in Antarctica and probably New Zealand (Maurin and Raoult, 1999).

The reservoirs of Coxiella burnetii are extensive. It has been found in various wild and domestic mammals, arthropods and birds (Angelakis and Raoult, 2010). Transmission of bacteria among wild animal occurs mainly through tick bite whereas C. burnetii infection in farmed ruminants usually occurs after inhalation of contaminated aerosols or direct contact withbody fluids or secretions (milk, urine, faeces or birthing products [amniotic fluid, placenta]) frominfected animals. Clinical signs of infection rarely develop in infected livestock. Coxiella burnetii localizes to the uterus and mammary glands of infected animals (Babudieri, 1959).If the infected animal is pregnant, low birth weight or abortion sometimes occur (Marrie, 2007; Marrie *et al*., 1996). Inflammatory response in the myometrium of goats and metritisas a unique manifestation of the disease in cattle has been reported (Arricau-Bouvery and Rodolakis, 2005).

The most commonly identified sources of human infection are farm animals such as cattle, goats, and sheep (Fournier *et al*., 1998).Inhalation of infectious particles is the most important route of transmission from animal to human. Occasionally people can get Q feverby consumption of unpasteurized contaminated milk. People at greatest risk for infection are veterinarians, farmers, sheep and dairymen. Also, people who are exposed to animal products particularly hides and woolareat risk, probably through the inhalation of tick faeces.Ticks expel large number of C. burnetii with their faeces onto the skin ofthe animal host at the time of feeding.Only half of people infected with Coxiella burnetii show clinical signs that can be presented as an acute or chronic form.

More than 55 years ago, seropositivity of human, cow, sheep, goat and dromedary was determined in Iran (Kaplan and Bertagna, 1955).After that only a few studies have been performed on the prevalence of Q fever in animal population in south of Iran (khalili and sakhaee, 2009; Rahimi *et al*., 2009). Indeed prevalence, distribution and epidemiological aspects of coxiellosis are unknown in Iran.

We performed the present study to determine the seroprevalence of Q fever in commercial dairy herds in northeast of Iran.

Materials and methods

Study area

The present study was conducted in Khorasan Razavi province that is one of the the most important centers of producing dairy products located in the northeastern part of the country. It covers an area of 118854 km² and has a human population of 5,593,079 and industrialized dairy cattle population of 174000 (www.amar.org.ir). This province shares border with Afghanistan in the east and Turkmenistan in the north (Fig. 1). Khorasan Razavi province contains 19 counties and Mashhad is the capital of the province. About 400 Commercial dairy herds are registered in veterinary head office of the province. Most of them are located around Mashhad (capital of Khorasan Razavi province).Commercial dairy herds in this area are comprised predominantly of Holstein-Friesian cattle that calve throughout the year and re-bred using artificial insemination. Cows are housed in open-shed barns and milked three times daily. Herds are fed on total mixed rations; diets are based primarily on corn silage, alfalfa hay and concentrates.

Sampling procedure

The sample size of animals to be bled was computed according to the following equation:

$$n = \frac{(z_{1-\frac{\alpha}{2}})^2 pq}{d^2}$$

Where n being the required sample size,

$z_{1-\frac{\alpha}{2}}$ is the normal deviate (1.96) at 95% confidence level, p is the estimated prevalence, q = 1-p and d is the precision of the estimate. With p set at 0.2 and d at 0.05, a sample size of 246 was required.

2 stage cluster sampling (Thrusfield, 1995) procedure were used to select study samples. The first stage of the sampling was the herds, and the second stage was the individuals in that herds. 19 commercial dairy herds randomly selected from the list of dairy herdswhich were registered in the veterinary head office of Khorasan Razavi province. Selected herds were located in 9 different counties. In each selected herd, numbers of dairy cattle (depends on herd size) were randomly selected (Calves and heifers were not included in thestudy). Finally, 246 dairy cows were chosen for this study.

Blood samples (10ml) were collected from the jugular vein of each animal. For each cow, identification of cow, herd of origin and parity of dam was recorded.

Samples were transported on ice to the laboratory. The samples were centrifuged at 1800 g for 10 minutes to obtain the serum. Sera were stored in identified vial at -20 degree of centigrade until testing.

Serological test

The sera were tested for the presence of antibodies against Coxiella burnetii using CHEKIT Q-fever ELISA kit (Idexx Laboratories, Switzerland) according to the manufacturer's instructions. Positive and negative controlling sera were provided by the manufacturer. Results were expressed as a percentage of the optical density reading of the test sample (%OD) calculated as below:

$$\%OD = 100 \times (S - N) / (P - N)$$

The S, N and P are the OD values of the test sample, the negative and the positive controls, respectively.

Sera were considered to be ELISA-positive if they had a value of 40% or more, suspicious if the value was between 30% and 40%, and negative if the value was <30%. Re-analysing of suspect samples was recommended by the manufacturer.

A herd was considered to be positive if at least one of selected cows from the herd was positive.

Statistical analysis

Herd and animal level seroprevalence and 95% confidence interval was calculated. Chi-square and Fischer exact test were used to assess the association between the explanatory variables (herd sizes, location andparity) and seroprevalence of Coxiellosis. All statistical analysis were performed using SPSS statistical software version 16 (SPSS Inc., Chicago) and P-value less than 0.05 considered to be significant.

Results

A total of 55 dairy cows were seropositive and 15 herds revealed at least one seropositive animal. Prevalence of antibody against Coxiella burnetii at animal and herd level was 22.3 (95% CI: 17.1-27.6) and 78.9 (95% CI: 60-97) percent respectively. 8(42%) of commercial dairy herds had a Coxiella burnetii seroprevalenceof more than 20%.

Seroprevalence of Coxiella burnetii in dairy cattle population with attention to herd size, parity, and location were presented in Table1. There was no association between the likelihood of seropositivity and parity of dam. Also, seroprevalence of Coxiella burnetii was not statistically associated with herd size. Except one, we found at least one positive sample in all counties. The seroprevalence rateranged from 0 to 62.5% and it was different significantly in various regions (p=0.001) (Figure 2).

Discussion

The results of the present study provide a useful insight into the prevalence and distribution of Coxiella Burnetii infection in Holstein dairy cattle in northeast of Iran. 22.3% of serum samples were seropositive. Infection with Coxiella Burnetii causes

economic loss in farm animals. Furthermore, farm animal has been identified as the most important source of human infection.High proportion of seropositive cows in the commercial dairy herds implies economic and public health importance of this infection in the study area.

Table 1. Animal level SeroprevalenceofCoxiellaburnetii with respect to parity, district and hers size.

Variables	Levels	No. of animal tested	Seropositive, N(%)	95% confidence interval	P-value
Parity					
	Primiparous	64	12 (18.8%)	(8.9-28.6)	P=0.421
	Multi parous	182	43 (23.6%)	(17.4-29.8)	
District					
	Mashhad	102	27 (26.5%)	(17.8-35.2)	
	Ghoochan	20	2 (10%)	(0.0 -24.4)	
	Taybad	16	10 (62.5%)	(35.9-89.1)	
	Torbatheydarieh	20	5 (25%)	(4.2-45.8)	
	Gonabad	16	2 (12.5%)	(0.0 -30.7)	P=0.001*
	Torbat jam	18	0 (0%)	(0.0 – 0.0)	
	Kashmar	13	2 (15.4%)	(0.0 -38)	
	Sabzevar	20	5 (25%)	(4.2-45.8)	
	Neishaboor	21	2 (9.55%)	(0.0 -23.2)	
Herd size					
	<100	33	5(15.2%)	(3.0 -27.4)	
	100-200	80	20 (25%)	(11.6-38.4)	P=0.519
	≥200	133	30 (22.6%)	(15.5-29.7)	
total		264	55 (22.4%)	(17.1-27.6)	

* Statistically significant.

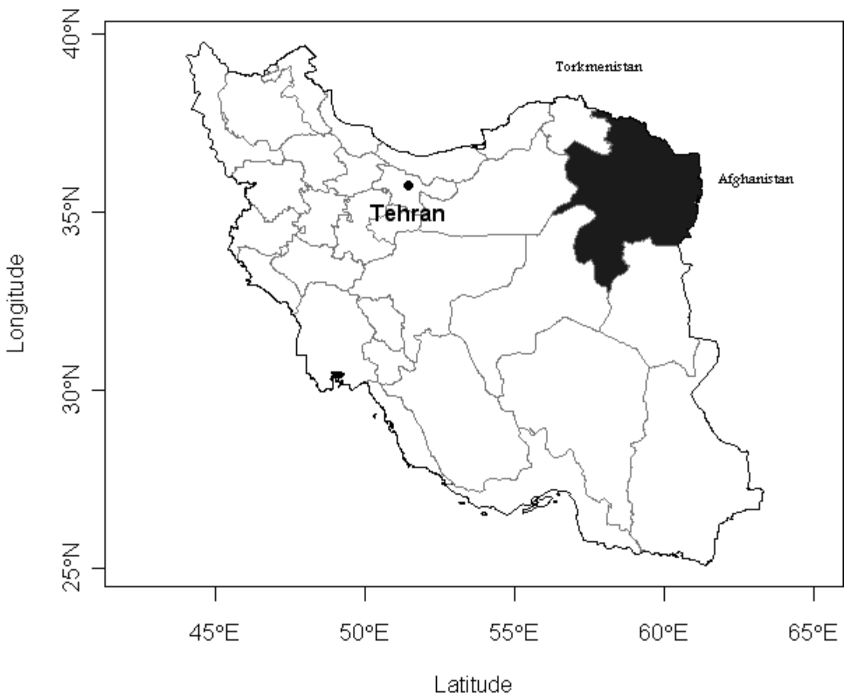


Figure 1. Map of the provinces of The Islamic Republic of Iran showing the location of KhorasanRazavi province (gray colour).



Figure 2. Geographical distribution of animal Levelseroprevalence (ALS) and herd level seroprevalence (HLS) in KhorasanRazavi province. Values shown as ALS are number of positive animals divided by total number of tested animals and for HLS are number of positive herd(s) divided by total number of tested herd(s).

Numerous studies have been conducted to evaluate the serological prevalence of coxiellosis in farm animals. Seroprevalence of Coxiella Burnetii had been various in different partsof the world. Animal level of seroprevalence of CoxiellaBurnetii in dairy cows was 6.2, 7.8,14.3, 14.5, 22.7 and 24% in five studies conducted in Northern Ireland, Germany,Central African Republic, Mexico and Cyprus, respectively (McCaughey, *et al.*, 2010; Psaroulaki *et al.*, 2006; Nakoune *et al.*, 2004; Hellenbrand *et al.*, 2001; salman *et al.*, 1990). Herd level of seroprevalence was 57% in Mexico and 48.4% in Northern Ireland (McCaughey, *et al.*, 2010; salman *et al.*, 1990). Also, seropositive human and animals are reported from countries around Iran. In Turkey (north-western neighbour of Iran) 20% of sheep were seropositive and 81% of flocks revealed at least one seropositive animal (Kennerman *et al.*, 2010). Another study showed that 4.3% of cattle serum samples were found PCR positive for Coxiella burnetii (Kirkan *et al.*, 2008). In Oman, located in south of Iran, 9.8% of adult patient and 52% of goats had been reported to be infected (Scrimgeour *et al.*, 2003).

Recent studies in southern parts of Iran showed animal level of seropositivity of 10.75, 65.78 and 29.42% for bovine, goat and sheep, respectively. They reported 100% of sheep and goat flocks and 16.6% of cattle herds had at least one positive member (Sakhaee and Khalili 2010; Khalili *et al.*, 2009). Another study on bulk milk by Nested PCR that was performed in Iran showed that 17.9% of dairy

herd were positive. They reported that none of ovine bulk milk sample and only 1(1.8%) of caprine bulk milk were positive (Rahimi *et al*., 2009). According to the results of present study, prevalence of antibodies against Coxiella Burnetii in the population of dairy cattle in northeast of Iran is higher than southern part of country and other parts of the world.

Present study showed that antibody against Coxiella is distributed all over the province. Although seroprevalenceof Q fever was different in various counties, but the number of herds sampled per county is lowto discuss the geographical diversity of seroprevalences. The herd selected for sampling in Taybad, one of the eastern counties of the province was highly seropositive. This herdis located near to the Iran-Afghanistan border. Although there is no information about seroprevalence of antibody against Coxiellaburnetii in domestic animals in Afghanistan but infection with Coxiellaburnetii among US soldiers deployed in Afghanistan confirmed presence of CoxiellaBurnetii in this country (Hartzell *et al*., 2007). Also, the climate of eastern part of Khorasan Razavi province is arid and semi arid. The dry atmosphere might enhance the dispersion of aerosols (Nakoune *et al*., 2004).

In present study, herd size and parity was not statistically associated with seroprevalence. McCaughey *et al*. (2010) performed a serological test on 5182 cows from 273 herds in Northern Ireland. They reported that Large dairy herds (>100 animals) showed higher seroprevalence at animal level than small herds (<50 animals). Also, Animals aged >2 years had significantly higher odds of seropositivity than animals aged<2 years (McCaughey, *et al*., 2010).Out of 19 selected herds in our study, none of them had herd size of less than 50 and only 4 herds had herd size of less than 100 animals. Also, all dairy cows entered to the present study had experienced at least one parturition that means that most of them were more than 2 years old while sampling. The results of the present study show that seroprevalence of Coxiellosis doesn’t change significantly after the first parturition.

In conclusion; present study has demonstrated a relatively high prevalence of current or past infection in the cattle population in the study area which has clear zoonotic and economic implications. Proper hygiene, especially during parturition needs to be considered to prevent animal to animal spread of disease. Raising awareness of the people who work with animals, immunodeficient patients or those suffering from cardiac valvuopathy and pregnant women is important.There is a remarkable diversity in the epidemiology of Q fever in different geographical regions (Norlander, 2000).Therefore, more investigations on the other reservoirs and human (especially at risk population) are necessary to make epidemiologic feature of Coxiellosis clear in the study area.

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سرواپیدمیولوژی بیماری تب کیو
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چکیده

بیماری تب کیو یکی از بیماریهای مهم مشترک بین انسان و دام است که توسط باکتری به نام Coxiella Burnetti ایجاد مـی گـردد. مهمترین منبع عفونت برای انسان دامهای اهلی از قبیل گاو، گوسفند و بز شناخته شده است. اطلاعات بسیار کمی درباره شیوع این بیمـاری در جمعیت دامی ایران وجود دارد. جهت بررسی شیوع سرمی بیماری در گاوهای شیری هلشتاین استان خراسان رضوی، تعداد ۲۴۶ راس گاو شیری از ۱۹ گله که در ۹ شهر مختلف استان قرار داشتند به روش نمونه گیری تصادفی خوشه ای انتخاب شدند و نمونـه سـرم خـون ایـن دامها از نظر وجود آنتی بادی بر علیه عامل این بیماری توسط کیت الیزای تب کیو ساخت شرکت Idexx سوییس مورد ارزیابی قرار گرفت. تعداد ۵۵ راس (۲۲/۳٪) از دامهای مورد بررسی از نظر سرمی مثبت بودند. همچنین در ۱۵ گله (۷۸/۹٪) از گله های مـورد مطالعـه حـداقل یک نمونه سرمی مثبت مشاهده شد.تیتر سرمی مثبت در گله های اکثر نقاط استان مشاهده شد. شیوع سرمی در گله های مختلف دامنه ای بین ۰ تا ۶۲/۵٪ را نشان داد و این نسبت در مناطق مختلف به صورت معنی داری متفاوت بود ($p=0.001$). سایز گله و تعداد شـکم زایـش ارتباط معنی داری با شیوع سرمی این بیماری نشان نداد. شیوع سرمی نسبتا بالا در جمعیت گاوهای شیری این منطقه، بر لزوم توجه بیشـتر به این بیماری مشترک تاکید می کند. بررسیهای بیشتر روی سایر دامهای مخزن این باکتری و نیز افراد در معرض خطر جهت روشن شدن جنبه های اپیدمیولوژیک بیماری ضروری است.

واژگان کلیدی: تب کیو، گاو شیری، شیوع سرمی